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GABA Binding in Human Blood Platelets: *In vitro* Effects of Lithium and Rubidium Chlorides

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Methodologic and kinetic properties of γ-aminobutyric acid (GABA) binding were studied in platelet materials of different nature. Platelet GABA binding was saturable and specific. LiCl and RbCl added *in vitro* at concentration of 0.01 mM increased significantly platelet GABA binding. This activator effect, and its kinetic characteristics were different from those obtained for GABA uptake and 5-hydroxytriptamine (5-HT) binding, which suggest that the three processes are different in platelets.

γ-aminobutyric acid (GABA) appears to be the major inhibitory neurotransmitter in the mammalian central nervous system (CNS) (2, 12, 19) and deficits of GABA have been implicated in certain neurological and psychiatric disorders, such as Huntington's chorea, Parkinson's disease, epilepsy, ansiety, schizophrenia (16) and affective disorders (6, 9). Some of the components of the brain GABA-system are also present in the peripheral nervous system, where GABA is not a transmitter. This first came to light in experiments on mammalian sympathetic ganglia, where the normal transmitter is acetylcholine.

Since it is known that blood platelets contain several neurotransmitter receptors such as dopamine (DA), noradrenaline (NA) and 5-hydroxytryptamine (5-HT) (5, 11, 13, 17) and blood platelets are able to accumulate GABA by a specific and high affinity uptake process (7), we investigated the possible existence of a GABA binding process by platelets.

Moreover, since the GABA has recently been implicated in affective psychiatric disorders in which lithium salts have a well established therapeutic effect and rubidium salts have recently demonstrated an antidepressive effect (4), we have examined the effect *in vitro* of lithium and rubidium chlorides on these possible GABA platelet receptors.

Materials and Methods

Blood was obtained by venipuncture from healthy volunter donnors of either

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sex between the age of 26 and 51 years. The blood was taken in a polyethylene syringe with ACD-A (1) as anticoagulant. Polyethylene tubes and polyethylene pipettes were used throughout.

Platelet materials

Platelet rich plasma (PRP) was obtained by centrifugation for 8 minutes at 300 × *g* in a refrigerated centrifuge Sorvall RC-5 at 15° C. Subsequent platelet isolations were made by discontinuous dextran gradients (P_D) according to GRAF *et al.* (10) or by gel filtration platelets (GFP) according to LEVY-TOLEDANO *et al* (15) technique. Platelet membranes (P_M) were obtained by streptolysin O rupture in tyrode-tris buffer pH = 6.5 at 37° C and subsequent ultracentrifugation at 15,800 × *g* in a L5-50 Beckman ultracentrifuge. Platelet counts were made by means of a Contrast Phase Microscopy. In each case, platelets counts were made in triplicate utilizing 10 μl aliquots of suspended platelets diluted in 2 ml of 1% ammonium oxalate. The platelet preparations were resuspended in tyrode-tris modificated buffer at pH = 7.4 (8) by rapid mixing. These preparations were observed under contrast phase microscopy and was not displayed platelet aggregation.

GABA binding

Binding of [^{3}H]-GABA was determined by incubating, in triplicate, 100 μl of samples containing a variable number of platelets previously fixed resupended in tyrode-tris buffer at pH = 7.4 with 50 μl of various concentrations of [^{3}H]-GABA between 2.5 × 10^{-8} M and 10^{-10} M final concentration. Incubation were carried out at 4° C to inhibit GABA uptake during a previously fixed time. Samples were preincubated before binding studies during 10 minutes at the same conditions. Following incubation, 1 ml of ice cold saline solution was added, and radioactive platelet pellets were quickly isolated by filtration on Whatman GFC filters. Filters were washed twice and then transferred to scintillation vials to which 10 ml of counting fluid was added. Platelet radioactivity was determined by liquid scintillation spectrometry using a Packard Tri-Carb counter model 2425. Counts were determined with internal standardization by the sample channels method. Non-specific binding was determined in parallel experiments by addition of unlabeled 10^{-6} M GABA. This amount was subtracted from total binding to obtain an estimate of specific GABA receptor binding.

In vitro *effects of LiCl and RbCl*

When the effects of lithium and rubidium were studied, LiCl and RbCl replaced to equimolar quantities of NaCl from the suspension medium. This replacement does not suppose any change in either pH or osmolarity of medium. Preincubation and addition of Li and Rb salts was preformed together with labeled-GABA in order to avoid possible effects of ionic interchange and others. The assayed [^{3}H]-GABA concentration was of 10^{-8} M and of unlabeled-GABA 10^{-6} M.

Results

On figure 1 is shown the effect of incubation time on different platelet materials. It is observed that when P_D or M_P were used there was a saturable kinetic of binding in respect to the time, but when PRP was used, specific [^{3}H]-GABA binding was very low and decreased with the incubation time.

The idoneus platelet number was of 10^5 platelets/mm^3 for PRP, decreasing at greater concentrations and it was of 4 × 10^5 platelets/mm^3 for P_D (fig. 2). The

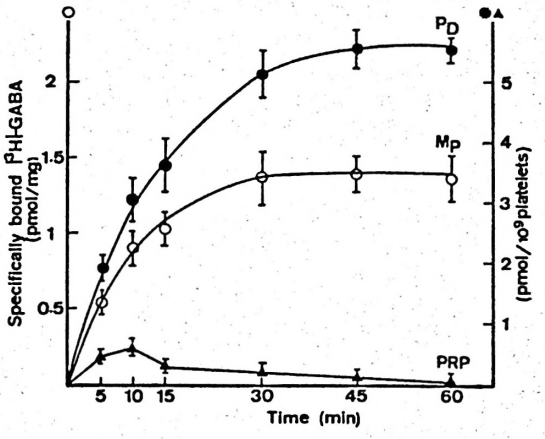

Fig. 1. *Time dependence of specific [³H]-GABA binding of platelet materials of different nature.*
Different platelet materials (▲) PRP, (●) P_D (10^5 platelets/mm³) and M_P (O) (0.5 mg of protein/ml) were incubated for various times at 4° C in tyrode-tris modified buffer, pH = 7.4, containing 10^{-8} M [³H]-GABA in the presence and absence of 10^{-6} M GABA as described in «materials and methods». The points are the means ± SD of three experiments, each preformed in triplicate.

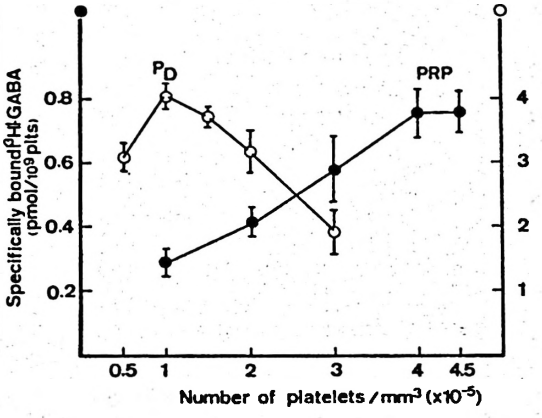

Fig. 2. *Platelet number dependence of specific [³H]-GABA binding.*
Various amounts of (●) PRP or (O) P_D were incubated for 10 minutes (PRP) and 30 minutes (P_D) at 4° C in tyrode-tris modified buffer, pH = 7.4. Points represent the mean ± SD of three experiments, each preformed in triplicate. Note that the different scales are expressed in the same units (pmol/10^9 platelets) in 10 minutes (PRP) and 30 minutes (P_D), respectively.

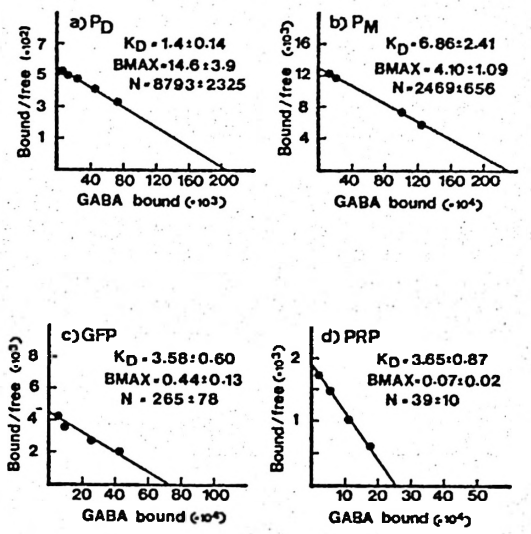

Fig. 3. *Scatchard plots of (³H)-GABA binding in different platelet materials.*
Different platelet materials: dextran isolated platelets (P_D), platelet membranes (P_M), gel filtred platelets (GFP) and platelet rich plasma (PRP), were incubated in tyrode-tris buffer at pH = 7.4 containing increasing concentrations, between 5×10^{-10} M and 2.5×10^{-8} M, of ³H -GABA in the absence and presence of 10^{-6} M of GABA, as described in «materials and methods». «K_D» are expressed as nM concentrations. «Bmax» are expressed as pmol of [³H]-GABA/10^9 platelets, and «N» as number of sites /platelet. Values represent the mean ±SD of three experiments, each preformed in triplicate.

Table I. *Effect of unlabeled 5-HT on |³H|-GABA binding by platelet membranes.*
Suspensions of membrane platelets in tyrode-tris buffer pH 7.4, were tested in binding assay with 10^{-6} M GABA and 10^{-4} M 5-HT, during 30 minutes, under conditions described in «Materials and Methods» at 10^{-8} M |³H|-GABA concentration in the incubation medium. Values represent the means ± SD of two experiments, each preformed in triplicate.

Conditions	cpm	% desplacement
10^{-8} M |³H|-GABA	2438.5±48	—
+10^{-6} M GABA	671.5±73	72.5±7.9*
+10^{-4} M 5-HT	2361.5±29	3.2±0.04**

* $p < 0.001$; ** N.S.

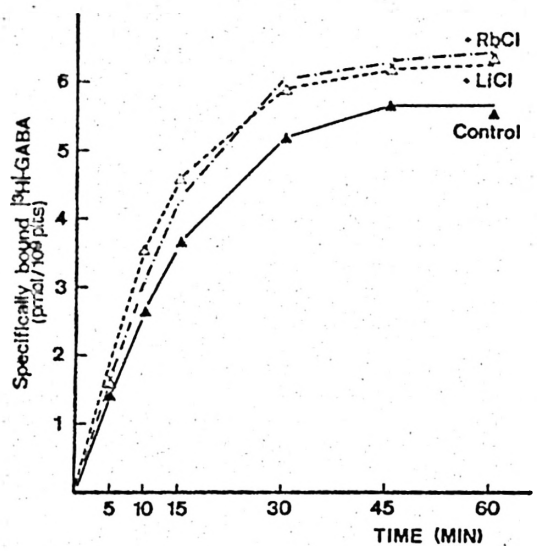

Fig. 4. *Effects of LiCl and RbCl on GABA binding on dextran isolated platelets*

Control platelet GABA binding, before (▲—▲) and after addition «in vitro» of 0.01 M of LiCl (Δ—Δ) and 0.01 M of RbCl (O— · O). Points are means for three experiments, each preformed in triplicate. The SD was less than 3-10% of mean values. The assayed [^{3}H]-GABA concentration was of 10^{-8} M.

equivalency between platelet number and mg of proteins is 10^9 platelets/ml is equivalent to 9.2 mg of proteins/ml. These amounts of platelets were used in all binding experiments.

Under these conditions it was assayed the effect of different concentrations of [^{3}H]-GABA from 2.5×10^{-8} M to 10^{-10} M. Scatchard analysis of binding data derived from experiments using different platelet preparations yielded the binding site characteristics shown in figure 3.

As platelets are considered as a 5-HT reservoir, the effect of this neurotransmitter on GABA binding was assayed. It was found that a concentration of 10^{-6} M of unlabeled 5-HT was not able to displace GABA from its binding sites (table I), while similar concentration of unlabeled GABA displaced it in about 73%.

When lithium and rubidium chlorides were added *in vitro* at 0.01 mM concentration (a dose that provided a marked reduced or increased platelet 5-HT binding) (18), they increased significatively the GABA binding by platelets at all assayed times (fig. 4). Comparations between LiCl and RbCl effects were not significative.

Discussion

Results obtained with ligand-binding have revealed the existence of a GABA binding in human blood platelets. We characterized this GABA binding by studying different parameters in order to obtain the optimal conditions for each assay. The specificity of GABA binding from different platelet materials was demonstrated by suppresion of [^{3}H]-GABA ligation by saturating concentrations of unlabeled GABA.

Platelet GABA binding was saturable in function of time when platelet membranes and dextran isolated platelets were used, but with platelet rich plasma the GABA binding increased until 10 minutes and then diminished with increasing time.

The Bmax and number of sites of platelet GABA binding was higher in P_D and diminished in direct relation in P_M, GFP and PRP, respectively. The low Bmax in GFP and PRP could possibly be due to the presence of a binding inhibitor in plasma. The K_D found in all materials indicate that GABA binding in platelets is highly specific (which demonstrates it could be a real GABA binding) although its physiological signification is not yet explainable.

There are also enough reasons to believe that platelet GABA binding is different to the 5-HT binding. Our hypothesis is based on two reasons: 1) 5-HT is not able to displace GABA from their binding sites, and 2) the effect of salts, subject of our studies, LiCl and RbCl, have different effects on both binding systems.

Our data also seems to demonstrate that these GABA binding sites are differ-

ent from those of GABA uptake by platelets since the affinity constants and the LiCl and RbCl effects *in vitro* are different. So, LiCl and RbCl increased significatively platelet GABA binding, while these have no effect on platelet GABA uptake.

Further evidence is necessary to confirm and characterize the pharmacological properties and biological relevance of these preliminary results about the existence of platelet GABA binding sites.

In the brain the GABA binding is correlated with its inhibitory neurotransmission but in platelets it is not understood in terms of its neurotransmission action. Indeed, several authors have reported the presence of extrasynaptic GABA systems, among them extrasynaptic receptors, but they consider them as a protective device against untoward effects of «overspilled» GABA on neural excitability (3).

Although extrasynaptic receptors for neurotransmitters are not uncommon, those for GABA are unusual in the sense that there are not comparable peripheral receptors for the other amino acid transmitters glycine, glutamate, or aspartate. It may also be of some significance that, in unmyelinated fibres, the two major extrasynaptic receptors —for GABA and acetilcholine respectively— mediate increases in Na and Cl^- permeability respectively, rather as though they are (or were) intimately concerned with the general control of cationic and anionic permeability, of which their synaptic function forms a specialization. Irrespective of its ultimate importance, the peripheral GABA-ergic system has provided a useful model for studying the action and transport of GABA (3).

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Resumen

Se estudian las características metodológicas y cinéticas de la fijación del ácido γ-aminobutírico (GABA) por materiales plaquetares de diferente naturaleza. La fijación de GABA muestra ser un proceso saturable y específico. Los cloruros de litio y rubidio añadidos *in vitro* a concentración de 0,01 mM, incrementan significativamente la fijación de GABA por las plaquetas. Este efecto activador, así como las características cinéticas del proceso, son diferentes a los encontrados para la captación de GABA y la fijación de 5-hidroxitriptamina (5-HT), lo cual sugiere que en las plaquetas sanguíneas los tres procesos son diferentes.

References

1. ASTER R. H. and JANDL, J. H.: *J. Clin. Invest.*, 43, 843-869, 1964.
2. BARBER, R. P., VAUGHN, J. E., SAITO, K., MCLAUGHLIN, B. J. and ROBERTS, E.: *Brain Res.*, 141, 35-55, 1978.
3. BROWN, D. A.: *Tins*, 2, 271-273, 1979.
4. CARROL, B. J. and SHARP, P. T.: *Science*, 172, 1355-1357, 1971.
5. DREUX, C., LAUNAY, J. M., DARGENT, A., PASQUES, D. and OSET-GASQUE, M. J.: *3rd World Congress of Biological Psychiatry*. Stockholm, 1981. F 108
6. EMRICH, H. M., ZERSSEN, D. V., KISSLING, W., MÖLLER, H. J. and WINDORFER, A.: *Arch. Psychiat. Nervenkr.*, 229, 1-16, 1980.
7. ENNS, L. and MCCOY, E. E.: *Br. J. Pharmac.*, 71, 553-556, 1980.
8. GADD, R. E. A., and CLAYMAN, S.: *Experientia*, 28, 719-720, 1972.
9. GOLD, B. I., BOWERS, M. B., ROTH, R. H. and SWEENEY, D. W.: *Am. J. Psychiatry*, 137, 362-364, 1979.
10. GRAF, M., LAUBSCHER, A., RICHARDS, J. G. and PLETSCHER, A.: *J. Lab. Clin. Med.*, 93, 257-265, 1979.
11. JAKOBS, K. H., SAUR, W. and SCHULTZ, G.: *Arch. Pharmacol.*, 302, 285-291, 1978.
12. KIM, J. S., BAK, I. J., HASSLER, R. and OKADA, Y.: *Exp. Brain Res.*, 14, 95-104, 1971.
13. KIM, B. K., STEINER, M. and BALDINI, M. G.: *Analyt. Biochem.*, 106, 92-98, 1980.

14. KLOTZ, I. M. and HUNSTON, D. L.: *Biochemistry*, **10**, 3065-3069, 1971.
15. LEVY-TOLEDANO, S., RENDU, F., BESSON, P. and CAEN, J.: *Eur. J. Clin. Biol. Res.*, **17**, 513-518, 1972.
16. LLOYD, K. G.: In «Enzymes and Neurotransmitters in Mental Disease» (Usdin, F., Sourkes, T. L. and Youdim, M. B. H. eds.) John Wiley. New York 1980. pp. 329-344.
17. MCMILLAN, R., BAKICH, M. J. and YELENOSKY, R. J.: *Br. J. Hematol.*, **41**, 597-604, 1979.
18. OSET, M. J. and CULEBRAS, J. M.: *An. Real Acad. Farm.*, **49**, 293-308. 1983
19. ROBERTS, E.: In «GABA in nervous system function» (Roberts, E., Chase, T. N. and Tower, D. B. eds.). Raven Press. New York 1976, pp. 515-529.